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Advances in emerging treatment options to prevent bronchopulmonary dysplasia

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ABSTRACT

Introduction: Bronchopulmonary dysplasia (BPD) is common sequela of premature birth.

It can be complicated by the development of pulmonary hypertension (PH), which significantly increases mortality. **Areas covered:** The aim of this review is to evaluate emerging drug therapies for prevention and treatment of BPD and associated PH. These include superoxide dismutase, macrolides, Clara secretory cell protein, α -1 protease inhibitors, pentoxifylline, melatonin, inositol, N-acetyl cysteine, allopurinol, cimetidine, pulmonary vasodilators and mesenchymal stem cells (MSC). We have also included discussion on longer standing therapies such as corticosteroids, caffeine and vitamin A.

Expert opinion: Corticosteroid administration systemically, but not by inhalation, caffeine and vitamin A reduce BPD. Enhancing endogenous anti-oxidant mechanisms through supplementation with superoxide dismutase or Clara cell secretory protein has yielded encouraging results. Azithromycin, a macrolide used to treat *Ureaplasma* infection, also has anti-inflammatory properties and has been shown to reduce BPD. Sildenafil reduces the echocardiographic markers of pulmonary hypertension associated with BPD, but has not been shown to prevent BPD. In animal models and a small phase one study MSC administration has resulted in promising results. Appropriately powered studies with long term outcomes are required to assess the efficacy of all these treatments.

1. Introduction

Bronchopulmonary dysplasia (BPD) is the most common adverse outcome of very premature birth and is associated with chronic respiratory morbidity through to adulthood [1] and increased mortality, particularly if it is associated with the development of pulmonary hypertension. Affected infants have a poor prognosis with a mortality of 47% within two years following the diagnosis of pulmonary hypertension [2]. Between 25 and 40% of premature infants with BPD have concomitant pulmonary hypertension [3].

Despite advances in the care of premature infants, including the routine use of antenatal corticosteroids, postnatal surfactant and gentler ventilation techniques, the incidence of BPD has not significantly changed [4]. This may reflect the survival of more immature infants and the changing nature of the disorder. BPD was first described in relatively mature, prematurely born infants who had severe respiratory distress syndrome and had received high concentrations of inspired oxygen and high ventilator pressures [5]. Histological specimens highlighted prominent fibroproliferation. Subsequently, very immature infants who had received minimal or no respiratory support in the first week after birth went on to develop oxygen dependency at 28 days, so-called “new BPD”. Their lung histology showed more homogenous changes, but with arrest of normal alveolar and vascular development [6].

Various definitions have been used to diagnose BPD including supplementary oxygen dependency at 28 days or 36 weeks post conception age (PCA). Comparison of use of the 28 day or 36 week PCA criteria has demonstrated, not surprisingly the later results in smaller percentage of infants being diagnosed with BPD, in one series 17% versus 40% [7]. An NIH consensus defined BPD as a requirement for supplementary oxygen at 28 days age [8]. Then, in those born before 32 weeks of gestational age, the severity of BPD severity was graded

according to respiratory support requirement at 36 week PCA, which better predicted long term respiratory outcomes [9]. Comparison of that definition with oxygen dependency at 36 weeks PCA or use of a room air challenge at 36 weeks PCA (a physiological definition of oxygen dependency) demonstrated variation in the proportion of infants diagnosed with BPD from 32% to 58.6%. The physiological definition was associated with lowest proportion of infants diagnosed [10]. Using an oxygen requirement at any gestational age to diagnose BPD is complicated by the increasing use of non-invasive respiratory support such as humidified high flow nasal cannula (HHFNC). Infants may be on air on HHFNC, but without HHFNC require supplementary oxygen. The criteria on which to diagnose BPD requires urgent revisiting with greater attention to capturing chronic respiratory morbidity. Compared to either 28 weeks or 36 weeks PCA criteria, a one year assessment of respiratory morbidity better predicted ongoing respiratory morbidity [11]. For the purpose of this review, pragmatically studies which used a supplementary oxygen requirement at 28 days or 36 weeks PCA to diagnose BPD were included [9].

Our aim was by reviewing the evidence base to determine which emerging therapies might reduce BPD and the associated pulmonary hypertension and which were the most promising and should be studied in larger trials. We have first discussed the efficacy of more longstanding therapies such as corticosteroids, vitamin A and caffeine.

2. Longstanding therapies

2.1 Corticosteroids

Systemically administered corticosteroids reduce BPD whether given in the first 96 hours after birth [12], at 7-14 days [13] or after three weeks of age [14]. Unfortunately, they have important side-effects including in the first 96 hours an increased risk of gastrointestinal

haemorrhage and intestinal perforation, but more importantly increased developmental delay at follow-up. When corticosteroids are administered systemically at older ages, there is no significant increase in cerebral palsy but, unlike trials recruiting in the first 96 hours, crossover was allowed which may have biased the results. In view of the many side-effects of systemically administered steroids, other options have been explored including inhaled corticosteroids (see below) and low dose dexamethasone. In one RCT, the latter therapy was associated with an increase in extubation by 10 days but no significant reduction in BPD [15].

Meta-analysis was undertaken of three trials which compared the efficacy of inhaled to systemic corticosteroids in ventilator dependent infants who were very low birth weight ie < 1500 gms (VLBW) or had a gestational age \leq 32 weeks and had a postnatal age greater than two weeks. No significant differences regarding reduction in BPD or side-effects were demonstrated [16]. A meta-analysis of eight trials which included 232 infants evaluated inhaled steroid administration in the first week from birth in infants at high risk of developing BPD. Inhaled steroids did not reduce BPD and death, failure to extubate or the total duration of mechanical ventilation or oxygen dependency [17]. The Neonatal European Study of Inhaled Steroids (NEUROSIS) randomised trial compared the effects of inhaled budesonide to placebo administered in the first 24 hours in 863 infants born at 23 to 27 weeks gestation who required some form of positive-pressure support [18]. The intervention was delivered until the infants no longer required supplementary oxygen or positive pressure support or were a postmenstrual age (PMA) of 32 weeks. Inhaled budesonide administration was associated with significant reductions in the rate of BPD, surgical closure of a patent ductus arteriosus (PDA) and reintubation , but there was a trend towards increased mortality (16.9% versus 13.6%) [18].

In-tracheal instillation of budesonide has been evaluated using surfactant as a vehicle. One hundred and sixteen VLBW infants with severe respiratory distress syndrome (RDS) and requiring mechanical ventilation and an inspired oxygen concentration of at least 60% were randomised to budesonide suspension with surfactant or surfactant alone [19]. The proportion of infants who survived without BPD was significantly higher in the budesonide group. There were no clinically significant adverse effects were reported [19] and follow at between two to three years demonstrated no significant differences in physical growth or neurological examination results [20]. A multicentre RCT was then undertaken including 265 infants with severe RDS. The budesonide group had a lower incidence of BPD or death (RR 0.58, 95% CI 0.44-0.77) and the number needed to treat was 4.1 [21].

2.2 Caffeine

The Caffeine for Apnoea of Prematurity (CAP) trial randomized infants if their clinicians thought them to be candidates for methylxanthine therapy for prevention or treatment of apnoea and/or facilitation of removal of an endotracheal tube in the first 10 days after birth. Two thousand and six infants with birth weights between 500 to 1250 gm were recruited. The primary outcome of death or neurodevelopmental disability (NDI) was determined at 18 to 21 months. The incidence of death or NDI was significantly lower in the caffeine group (40.2% versus 46.2%) and who also had a lower BPD rate, a secondary outcome (36.3% versus 46.9%) [22]. At five years, there were no significant differences in survival without disability [23]. Methylxanthines inhibit adenosine receptors that have been linked to anxious and aggressive behavior in mice with targeted deficiencies of each of those receptors [24, 25], thus the results of the CAP trial are reassuring. Caffeine acts by increasing central drive and by lowering the threshold of response to hypercarbia as well as by stimulating diaphragmatic contractility and prevention of a phragmatic fatigue, it also has a diuretic action [26].

Caffeine use was associated with a lower PDA incidence likely due to the increased diuresis and improved cardiac and pulmonary function [27], which may in part have been responsible for the reduction in BPD. It also has anti-inflammatory properties; for example in an animal model of hyperoxia caffeine reduced the influx of inflammatory cells into the lung and attenuated hyperoxia-induced upregulation of pro-inflammatory markers [28]. Caffeine may also inhibit Smad signaling and TGF- β 1 regulated genes in airway remodeling [29]. Post hoc analysis of the CAP trial has demonstrated infants receiving respiratory support appeared to derive more neurodevelopmental benefit from caffeine. Early initiation of caffeine therapy may be associated with a greater reduction in ventilation duration [30].

2.3 Vitamin A

Vitamin A is a group of fat-soluble compounds which maintain the integrity of epithelial cells of the respiratory tract. In a lamb model of vitamin A deficiency, histopathological changes included necrotizing tracheobronchitis and squamous metaplasia. Restoration of vitamin A to replete levels resulted in more normal surface epithelium in the conducting airways [31]. Premature infants are vitamin A deficient [32] and a prospective study demonstrated lower vitamin A levels in those who subsequently developed BPD [33].

A Cochrane review of vitamin A supplementation in very low birth weight infants or those born before 32 weeks of gestational age identified 11 trials including more than 1500 infants in total. Vitamin A administration was associated with a small, but significant reduction in BPD as diagnosed at 36 weeks PCA (RR 0.87, 95% CI 0.77 to 0.99), with the number needed to treat of 11 (95% CI 6 to 100) [34]. There was, however, considerable heterogeneity in the trials with regard to their inclusion criteria and intervention, including the birth weight, ethnicity, baseline vitamin A status, route of vitamin A supplementation (intramuscular,

intravenous in lipid emulsion, or enteral), and the vitamin A dose given. The only trial (n=807) reporting long term neurodevelopmental follow up to 18 and 22 months demonstrated no significant differences in outcomes [35]. It has been suggested that BPD might be reduced with the combination of vitamin A and inhaled nitric oxide [36]. The study, however, was not randomized and the data analysed retrospectively [36].

3. Emerging therapies

3.1. Superoxide Dismutase Replacement

Superoxide dismutases (SODs) catalytically convert the superoxide free radical to oxygen and hydrogen peroxide, hence protecting the lung tissue from oxidative injury caused by free radicals in a hyperoxic environment [37]. The enzymatic activity of SODs are similar in the premature and mature human lung [38]. Transgenic mice modified with knock-out of genes encoding for SODs had disruption of alveolar development at baseline and an exaggerated poor response to oxidative stress [39]. Conversely, transgenic mice which over-expressed human SODs, when exposed to hyperoxia had less pulmonary neutrophil influx and oxidized glutathione and preservation of alveolar surface and volume density compared with wild-type littermates [40]. In animal models of prematurity and pulmonary hypertension, treatment with intra-tracheal preparations of SODs protected against acute lung injury [41-43].

In a study of 33 infants with a birth weight between 600 and 1200g, those treated with intra-tracheal recombinant human CuZnSOD (rhCuZnSOD) every 48 hours for seven days had increased activity of the enzyme in their serum, tracheal aspirate and urine. Markers of lung injury in the tracheal aspirate were lower in the treated infants [44]. Subsequently, in a multi-centre randomised control trial (RCT), 302 infants were treated with either intra-tracheal rhCuZnSOD or placebo every 48 hours while they remained mechanically ventilated until a

postnatal age of 28 days. There were no statistically significant differences in BPD, death, or the combined outcome of BPD and death. In a subset born prior to 27 weeks of gestation, however, those treated with rhCuZnSOD received fewer asthma medications ($p=0.01$) and had lower rates of emergency room visits ($p=0.01$) and hospitalization ($p=0.05$) at follow up [45]. Despite those promising results, there have been no further clinical trials reported.

3.2. Macrolides

A systematic review highlighted a significant association between pulmonary colonisation with *Ureaplasma* and BPD [46]. Macrolides such as erythromycin, clarithromycin and azithromycin are used to treat *Ureaplasma* infections [47].

A systematic review was undertaken of randomised or quasi-randomised studies of either prophylactic or therapeutic erythromycin in preterm or low birth weight (LBW) infants [48] with either unknown *U. Ureaplasma* status or proven positive by culture or polymerase chain reaction (PCR). Only two small RCTs were identified both involving intubated infants less than 30 weeks of gestation; neither showed a statistically significant reduction in BPD or death or the combined outcome [48].

Clarithromycin [49], either prophylactically or in infants with *Ureaplasma* has not been shown to prevent BPD [50]. In animal models of colonization of the respiratory tract by *Ureaplasma*, both azithromycin and clarithromycin accumulated more in the lung epithelial lining fluid and alveolar macrophages than erythromycin [49, 51].

Azithromycin, however, might reduce BPD not only by its anti-microbial action against *Ureaplasma spp.*, but also via its immunomodulatory and anti-inflammatory activities. In

murine models of inflammatory lung disease, azithromycin reduced leukocyte infiltration of the lungs and inflammatory cytokines [52]. In addition, administration of azithromycin blocked release of pro-inflammatory cytokines (IL-6 and IL-8) in cells acquired from tracheal aspirates of preterm infants [53]. In a RCT of 33 infants with *Ureaplasma spp* as detected by PCR analysis of tracheal aspirates, azithromycin treatment was associated with a reduction in BPD (odds ratio (OR) 0.026, 95% confidence intervals (CI) 0.001 to 0.618) [54]. The dosage of azithromycin used in the study was 10mg/kg for seven days and then 5mg/kg subsequently for a maximum of six weeks [54]. Pharmacokinetic data, however, suggest that the optimal dose may be higher [55]. A systematic review of studies of prophylactic, intravenous azithromycin versus placebo included 310 infants born at less than 30 weeks of gestational age or of low birth weight. Azithromycin administration was associated with a reduction in BPD (relative risk (RR) 0.83, 95% CI 0.71 - 0.97) and the combined outcome of BPD and death, but no significant change in mortality [49]. In a subsequent systematic review of 11 trials which included 473 infants azithromycin was demonstrated to reduce BPD in extremely low birth weight (ELBW) infants (RR 0.83, 95% CI 0.72 - 0.98, p=0.02) [56]. There was no significant difference in the incidence of elevated liver enzymes, but there were four cases of hypertrophic pyloric stenosis [56].

Both azithromycin and erythromycin have been linked to the development of idiopathic hypertrophic pyloric stenosis, but the results are from a retrospective study. The association was strongest if the exposure occurred in the first two weeks after birth [57]. There are no reported cases of cardiac side effects as seen in adults, in infants treated with azithromycin [56].

3.3. Clara Cell Secretory Protein

Clara cell secretory protein (CCSP) is predominantly secreted by mucosal epithelial cells of the airway. CCSP is synonymous with Clara cell 10kD protein and CC16, which reflect its low molecular weight of 15.84kD. Endogenous CCSP has potent anti-inflammatory activity, inhibiting pro-inflammatory cytokines and neutrophil infiltration. In addition, it inhibits secretory phospholipase A2, an enzyme which degrades surfactant and facilitates prostaglandin biosynthesis [58]. Transgenic mice deficient in CCSP have been demonstrated to have exaggerated pulmonary inflammatory responses to hyperoxia and higher mortality rates [59]. Intra-tracheal treatment with exogenous CCSP tended to improve gas exchange and lung compliance in newborn piglets exposed to a hyperoxic injury [60]. A study in a premature lamb model found that treatment with synthetic recombinant human Clara cell secretory protein (rhCC10), increased surfactant protein and VEGF levels [61]. Co-administration of surfactant and rhCC10 significantly reduced RDS-induced lung and systemic inflammation in a lamb model compared to surfactant alone [62].

Levels of CCSP increase with increasing gestational age in human infants [63]. CCSP concentrations have been demonstrated to correlate negatively with the concentration of inspired oxygen required by preterm infants with RDS [64]. In a prospective study, CCSP levels in tracheal aspirates were lower from day one after birth in infants who developed BPD compared to those who did not [65].

One study has investigated the efficacy of intra-tracheal administration of rhCC11300. In 22 infants with birth weights between 700 and 1300 gms, there was no statistically significant reduction in BPD (RR 0.50, 95% CI 0.06 to 4.33) [64]. A Cochrane review identified only one pilot study [66]. A Phase II clinical trial (NCT01941745) is underway to investigate the

pharmacokinetics, safety, tolerability and anti-inflammatory effects of a single, intra-tracheal administered dose of rhCC10 [67].

3.4. α -1 Protease Inhibitor

Elastase is a proteolytic enzyme released as a consequence of oxidative damage by free radicals. α -1 Protease Inhibitor (α -1PI) inhibits elastase; endogenous levels are lower in prematurely born infants compared to those born at term and may be particularly low in those who develop BPD [68]. In a mouse model, genetically modified to express a human serine elastase inhibitor (elafin), compared to wild type controls there was a blunted inflammatory response to high supplementary oxygen and ventilator induced lung injury. This was indicated by NF- κ B activation, influx of neutrophils and monocytes, TGF- β activation and apoptosis and preserved matrix elastin [69].

Meta-analysis of two RCTs of α -1PI versus placebo which included a total of 195 infants, demonstrated a small, but statistically significant effect on 28 day oxygen requirement (RR 0.80 (95% CI 0.65 to 0.98)), but no statistically significant difference in oxygen requirement at 36 weeks PCA [70]. In one of the two trials, a secondary outcome of pulmonary haemorrhage was lower in the treatment group (RR, 0.22; CI, 0.05 to 0.98) which suggests α -protease may have further effects on the lung which could potentially contribute to BPD development [71]. No subsequent studies have been performed to verify those results.

3.5. Pentoxifylline

Pentoxifylline is a synthetic methylxanthine derivative. It has anti-inflammatory, anti-fibrotic and immunomodulatory properties. Unlike caffeine and theophylline, at a therapeutic dose pentoxifylline does not cause significant bronchodilation, central nervous stimulation, or

cardiac side effects [72]. It causes erythrocyte phosphodiesterase inhibition with down-regulation of inflammatory cytokines, including TNF- α and ICAM-1, which are implicated in the development of BPD [73].

Neonatal rats exposed to hyperoxic lung injury treated with pentoxifylline had reduced fibrin deposition (a correlate of severity of lung injury) and prolonged survival, although levels of TNF- α were unchanged [74]. Another study of treated neonatal rats demonstrated decreased lung oedema and macrophage infiltration, with increased lung anti-oxidant enzyme activity (superoxide dismutase, catalase and glutathione peroxidase). Additionally, pulmonary vascularization was improved with enhanced VEGF protein expression [75]. A model of volume induced lung injury in mechanically ventilated piglets showed a reduction in platelet activating factor (a proxy for inflammation) in the lungs of the pentoxifylline treated group. There was also a reduction in lung oedema and lung tissue myeloperoxidase activity, a measure of neutrophil presence [76].

The efficacy of pentoxifylline in the prevention of BPD has been evaluated in two clinical settings, prematurity and sepsis. In VLBW infants, a pilot RCT of nebulized pentoxifylline versus placebo with 100 participants showed a significant difference in the risk of BPD (OR: 0.32; CIs 0.11 to 0.94; $p = 0.039$). There were, however, methodological weaknesses in the RCT including the assessors were not blinded to treatment group and there was complete outcome reporting in only 65% of those recruited [77]. When the data were analysed on an intention to treat basis the differences no longer reached statistical significance [78]. A subsequent RCT of 80 infants with a gestational age less than 28 weeks, with more robust blinding and 100% outcome data, did not show a statistically significant difference in the incidence of BPD or the total duration of oxygen therapy ($p=0.75$) [79]. In the setting of

neonatal sepsis pentoxifylline, in addition to antibiotic therapy, did not reduce BPD (RR 1.50, 95% CI 0.45 to 5.05) [80].

3.6. Melatonin

Endogenous melatonin protects against oxidative stress both by direct scavenging of the highly reactive hydroxyl free radical and indirectly by modulation of anti-oxidant enzymes [81, 82]. Preterm infants are relatively melatonin deficient [83]. In rat models subjected to oxidative stress, melatonin treated animals showed an increase in anti-oxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) activity, reduction in neutrophil influx and a more normal alveolar architecture versus the interstitial fibrosis seen in untreated controls [84, 85].

A study of 110 infants born less than 32 weeks of gestational age with RDS and supported with a range of ventilation modes (conventional ventilation, pressure-support ventilation, volume guarantee and high-frequency oscillatory ventilation) found that those given melatonin had a reduction in inflammatory cytokines IL-8 and TNF- α , all associated with the development of BPD. Additionally, the treated group had significantly lower maximum inspired oxygen concentrations, peak inspiratory pressures, peak end expiratory pressures and oxygenation index [86]. In an extension of that study, the authors assessed 120 infants with RDS born at less than 32 weeks of gestation. No infants in the melatonin group, but eight infants in the control group developed BPD (this difference was not statistically significant). Those with BPD, however, had statistically significant higher levels of initial inflammatory markers (IL-6, IL-8 and TNF- α), which the group had previously shown to be ameliorated by melatonin treatment. No adverse effects of melatonin treatment were reported [87].

3.6. Inositol

Inositol, an essential six-carbon ring sugar, is a component of the group of phospholipids called phosphoinositides and glycosyl-phosphatidylinositols and is involved in surfactant maturation. Infants with a premature drop in inositol levels have been demonstrated to develop more severe RDS [88]. In a preclinical rat model, supplementation of inositol normalised phospholipid levels in deprived rat pups [89].

A Cochrane review of four RCTs which included 343 infants showed no statistically significant difference in BPD at 28-30 days with repeated doses of inositol versus placebo (RR 0.78, 95% CI 0.54 to 1.13) nor in BPD at 36 to 38 weeks PCA [90, 91]. In a study of 65 infants, however, who received a single dose of inositol, there was no statistically significant difference in the relative risk of BPD at 36 weeks PCA (RR (2.74, 95% CI 0.88 to 8.48; P = 0.08) [92].

3.8. Cysteine and N-acetyl cysteine

L-cysteine is a precursor to the anti-oxidant, glutathione. Deficiency in L-cysteine in the prematurely born infant may lead to increased susceptibility to an oxidative insult. Delivery of cysteine has practical issues due to solubility and stability, thus its more stable precursor N-acetyl cysteine is used [93].

A Cochrane review assessed the efficacy of supplementation of parentally fed infants with cysteine or N-acetyl cysteine with secondary outcomes of oxidative injury including BPD [94]. No studies reported on the impact of cysteine on BPD. N-acetyl-cysteine did not reduce BPD (n=328, RR 0.99, 95% CI 0.76 to 1.29), nor increase plasma levels of cysteine. In one study, the plasma concentration of cysteine was lower in the N-acetyl cysteine treated

group [95].

3.9. Allopurinol

Xanthine oxidase is implicated in the generation of oxygen free radicals under hypoxic-ischaemic conditions. Allopurinol is a xanthine oxidase inhibitor. In a RCT, 400 infants with a gestational age between 24 and 32 weeks were allocated to either a seven day course of allopurinol or placebo. At recruitment, the infants who subsequently developed BPD had significantly higher levels of hypoxanthine. There were, however, no statistically significant differences in BPD or other sequelae of prematurity between the two groups [96].

3.10. Cimetidine

Cimetidine is an inhibitor of the cytochrome P450 enzyme system. Cytochrome P450 facilitates mono-oxygenation reactions which produce oxygen free radical species. In a rat model, induction of cytochrome P450 reduced survival under oxidant stress. Furthermore in rabbits, inhibitors of cytochrome P450 significantly reduced lung oedema and prevented the reperfusion-related increase in lung microvascular permeability, but there was no reduction in free radicals in treated rabbits. In a lamb model of hyperoxic lung injury, cimetidine was demonstrated to be an effective protection against lung injury. In contrast to the rabbit model, the oxidized glutathione level was significantly lower in cimetidine-treated lambs, suggestive of reduced oxygen free radicals [97].

An RCT of intravenous cimetidine or placebo in 84 infants with a birth weight less than 1250g was stopped prematurely due to increased incidence of death and severe intraventricular haemorrhage in the treatment arm. At that point, there were no statistically significant differences in BPD or other outcomes [98].

4. Pulmonary vasodilators

Animal studies of placental insufficiency and prematurity have demonstrated derangement of the nitric oxide - cyclic guanosine monophosphate signaling pathway, which is associated with abnormal vascular responses. Both quantitative immunoblot analysis and semi-quantitative immunohistochemistry highlighted less endothelial nitric oxide synthase (eNOS) protein in the endothelium of small intrapulmonary arteries and epithelium of small airways of preterm lambs that had been mechanically ventilated for three weeks compared to lambs born at term [99]. Those results support the hypothesis that decreased eNOS in the pulmonary circulation and respiratory tract may contribute to the pathophysiology of BPD [99].

Pulmonary vasodilators then could have efficacy in the prevention of BPD.

4.1 Inhaled nitric oxide

Inhaled nitric oxide (iNO) selectively decreases pulmonary vascular resistance and hence improves oxygenation. It also has anti-inflammatory properties and promotes cell and blood vessel growth in the immature lung. Infants dying with BPD have been demonstrated to have disrupted pulmonary vasculature and hence it has been proposed that iNO might reduce BPD. Indeed in an animal model, iNO stimulated angiogenesis and alveolarisation [100].

Meta-analysis of 14 randomised trials demonstrated that the effect of iNO in prematurely born infants regarding prevention of BPD was dependent on the timing of administration and the population studied [101]. Nine trials of early rescue treatment of infants based on oxygen criteria demonstrated no significant effect of iNO on mortality or BPD. Similarly, routine use of iNO in infants with pulmonary disease demonstrated no significant reduction in BPD or mortality. One RCT, however, highlighted that early, prolonged, low dose iNO did not reduce BPD in infants who required invasive or non-invasive respiratory support [102] nor

did it improve outcomes at follow-up [103]. A further RCT [104] confirmed that iNO given non-invasively in the first 72 hours after birth did not reduce BPD. Infants receiving iNO all born less than or equal to 28 weeks of gestation and requiring mechanical ventilation at 7 to 14 days were randomised to receive up to five doses of surfactant or sham instillation every one to three days [105]. Although the surfactant administration was well tolerated it did not improve survival without BPD at 36 or 40 weeks. Inhaled nitric oxide has been given to infants with pulmonary hypertension and BPD with anecdotal success. iNO administration in a small series resulted in reduction in pulmonary artery pressures to near normal levels [106].

4.2 Sildenafil

Phosphodiesterase-V is highly expressed in the lung and metabolises cyclic guanosine monophosphate (GMP), a regulator of NO-mediated vascular relaxation. Sildenafil is a phosphodiesterase-V inhibitor and hence increases cGMP concentrations and results in pulmonary vasodilation. Sildenafil acts as a vasodilator both in the pulmonary vasculature and, to a lesser degree, systemically.

In rat models of hyperoxia-induced BPD, sildenafil administration improved alveolar growth and lung angiogenesis. Additionally, the animals treated with sildenafil had less right ventricular hypertrophy and reduced echocardiographic signs of pulmonary hypertension [107, 108]. In lambs with experimental pulmonary hypertension, both enteral and aerosolized sildenafil resulted in dilation of the pulmonary vasculature and augmentation of the pulmonary vascular response to iNO [109, 110].

There is limited information regarding the use of sildenafil to prevent BPD. In a pilot RCT of 20 infants, four weeks of oral sildenafil solution (3mg/kg/day) was compared to placebo

commenced on day seven in mechanically ventilated infants of less than 28 weeks of gestational age. There was no statistically significant difference in BPD at 36 weeks PCA, but a non significant trend for the sildenafil treated group to require more hours of mechanical ventilation and postnatal steroid treatment and they also had a higher respiratory-related mortality [111].

In pulmonary hypertension associated with existing BPD, small retrospective case series have demonstrated benefit from sildenafil with regard to echocardiographic assessments of pulmonary hypertension [3, 112, 113], but not always associated with clinical improvement [112]. One retrospective study with long term follow up of 22 infants showed that sildenafil was well tolerated with long term improvement in pulmonary hypertension, although 20% of the study population died [114]. In a recently reported, retrospective study, 12 of 18 infants with BPD and related pulmonary hypertension were treated with sildenafil, five received sildenafil and bosentan and another bosentan only [115]. The median follow-up was two years and the improvement in pulmonary hypertension was maintained at the last follow-up with a better survival rate (95%) than previously reported. The lack of control groups and/or limited follow up, however, reduces the generalisability of the results to clinical practice. In addition, the optimum dose of sildenafil has not been robustly established and the absorption of drug when given orally is variable in prematurely born infants.

4.3 Bosentan

Bosentan is an endothelin-A (ETA) and endothelin-B (ETB) receptor antagonist, which prevents potent vasoconstriction caused by endothelin 1 binding to ETA and ETB receptors. It has been used extensively in adult patients with pulmonary hypertension. There is no published evidence for its use in prematurely born infants at risk of BPD. A Cochrane review

of two small RCTs of bosentan in infants born after 34 weeks of gestation with persistent pulmonary hypertension concluded that there was inadequate evidence to support the use of bosentan either as stand-alone therapy or as an adjuvant to inhaled nitric oxide [116]. There are single case reports of the efficacy of bosentan in BPD associated pulmonary hypertension [117, 118].

4.4 Prostacyclin

Prostacyclin is produced in the vascular endothelium and causes smooth muscle relaxation. Pulmonary hypertension in the neonatal and paediatric population is associated with decreased prostacyclin synthesis [119]. The usefulness of prostacyclin administration such as epoprostanol, however, is limited by its short half life and hence a continuous infusion is required via a central line. Newer synthetic analogues such as iloprost and treprostinil have a longer half-life. There are no reports for its use as a primary preventative measure for BPD. The evidence for its efficacy in neonates with BPD associated with pulmonary hypertension is limited to case reports [117, 120].

5. Stem cells

There are some promising early results of the potential effect of stem cells on BPD. Hyperoxia induced BPD in neonatal rats is associated with decreased circulating and resident mesenchymal stem cells (MSC) [121]. Administration of bone marrow derived MSCs or multipotent stromal cells prevented the compromised alveolar and vascular development in a murine hyperoxia induced BPD model [121]. Treatment with MSC conditioned media after hyperoxia might then reverse the disease [122]. Bronchioalveolar stem cells (BASCs) are an adult lung stem cell population capable of self-renewal and differentiation in culture [123]. They proliferate in response to bronchiolar and alveolar lung injury in vivo [123]. Systemic

treatment of neonatal hyperoxia-exposed mice with MSC and MSC-conditioned media resulted in an increase in BASCs. The authors suggested MSCs and MSC-derived factors might stimulate BASCs to play a role in the repair of lung injury in BPD [123]. A source of stem cells is the human cord. In an oxygen induced model of BPD, human cord derived perivascular cells (PCs) or cord blood derived MSCs delivered into the airway of rat pups resulted in rescued lung function and structure. Cell engraftment was low suggesting the PCs and MSCs may act via a paracrine effect. Assessment at six months showed no adverse lung effects [124]. In a rat model, hyperoxia exposure led to air space enlargement, loss of lung capillaries and low expression of VEGF and eNOS. Transplanted endothelial progenitor cells, when combined with iNO, resulted in improved alveolarisation, microvessel density and upregulation of VEGF and eNOS proteins [125]. In a rat model of BPD induced by perinatal inflammation and hypoxia administration of MSCs improved vascular density and reduced TNF α , IL-6 and collagen density to normoxic levels [126]. Thus, MSCs attenuated perinatal inflammation and hypoxia induced defective alveolarisation and angiogenesis and reduced lung fibrosis [126]. A phase 1 dose-escalation trial has been undertaken to assess the safety and feasibility of a single, intratracheal transplantation of human umbilical cord blood (hUCB) derived MSCs in preterm infants at high risk of BPD. Nine infants, mean gestational age of 25.3 weeks were included in the trial at a mean of 10.4 days. The first three patients were given a low dose (1×10^7 cells/kg) and the next six a high dose (2×10^7 cells/kg). Comparison was made to historical case-matched infants. The treatment was reported to have been well tolerated and no serious side-effects were reported. Levels of IL-6, IL-8, TNF α , metalloproteinase-9 and transforming factor β 1 in tracheal aspirates at day seven were significantly lower than at baseline. BPD severity was lower in the transplant recipients [127]. Those promising results should be further investigated in appropriately designed studies.

6.5. Conclusion

There are several emerging therapies which reduced BPD or associated pulmonary hypertension, but none have been robustly tested in large RCTs with long term follow-up. Superoxide dismutase replacement has been shown in one trial to improve long term respiratory outcomes in an immature subgroup, but not to reduce BPD. Azithromycin does reduce BPD in confirmed *Ureaplasma* spp. infection and in the absence of positive isolates. Additional work, however, is needed to clarify the optimal dosage for neonates and determine whether there are no adverse gastro-intestinal side effects. Recombinant Clara cell protein and mesenchymal stem cells appear promising new therapies. There is insufficient evidence at present to support routine use of pentoxifylline, melatonin, inositol and N-acetyl-cysteine, allopurinol or cimetidine. Further studies are required to determine whether pulmonary vasodilators might prevent BPD and evaluate their efficacy in the management of infants with pulmonary hypertension associated with BPD.

Expert Opinion (Table)

Potentially interesting results have been demonstrated with the anti-oxidant, recombinant superoxide dismutase (SOD), but these have not encouraged other studies, perhaps as multiple doses of SOD had to be given intra- tracheally. In animal models, augmentation of the endogenous anti-inflammatory pathway with recombinant analogue of Clara cell secretory protein, rhCC10 has yielded promising results, but this has not yet been translated into important clinical outcomes. A phase one study of mesenchmal stem cells yielded promising results with no reported adverse effects. Only nine infants, however, were included and received a single dose given intracheally. This therapy merits further testing.

Azithromycin, which acts as both an anti-inflammatory and anti-bacterial agent with efficacy against *Ureaplasma*, reduced BPD when used prophylactically and in those with confirmed *Ureaplasma* colonization. Caution, however, is advised, as azithromycin has been associated with the development of hypertrophic pyloric stenosis. Meta-analysis of supplementation with vitamin A has demonstrated reduction in BPD, but no change in longer-term outcomes, further emphasising that BPD is not an appropriate outcome for RCTs assessing agents to prevent the chronic respiratory morbidity of prematurely born infants. Indeed, there should be focus on therapies which should reduce chronic respiratory morbidity than BPD. Infants with BPD associated pulmonary hypertension have a poorer prognosis. There is an urgent need to determine which pulmonary vasodilators might improve the outcome of those BPD infants with pulmonary hypertension.

ARTICLE HIGHLIGHTS

- Azithromycin reduces BPD when used prophylactically and in infants colonized with *Ureaplasma*
- In animal models of BPD recombinant Clara cell secretory protein reduced lung inflammation.
- An RCT of recombinant superoxide dismutase demonstrated improved long term respiratory outcomes, such as reduction in asthma medications and hospitalization with respiratory illness
- Meta-analysis highlighted vitamin A reduced BPD, but not long term outcomes and there was considerable heterogeneity of included trials.
- Sildenafil improved echocardiographic markers of pulmonary hypertension associated with BPD, but did not prevent BPD.
- Administration of mesenchymal stem cells have improved short term outcomes in

animal models and in a small phase one study.

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** In a phase-1 dose-escalation trial ↑ of mesenchymal stem cells with promising results*

Abbreviations:

Post conception age (PCA)

Bronchopulmonary dysplasia (BPD)

Relative risk (RR)

Confidence Interval (CI)

Superoxide dismutase (SOD)

Recombinant human CuZnSOD (rhCuZnSOD)

Clara cell secretory protein (CCSP)

Recombinant human clara cell protein (rhCC10)

α -1 Protease Inhibitor (a1PI)